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*Published in:*  
Eurosurveillance

*DOI:*  
[10.2807/1560-7917.ES.2015.20.41.30040](https://doi.org/10.2807/1560-7917.ES.2015.20.41.30040)

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*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2015

[Link to publication in University of Groningen/UMCG research database](#)

### *Citation for published version (APA):*

Bathoorn, E., Rossen, J. W., Lokate, M., Friedrich, A. W., & Hammerum, A. M. (2015). Isolation of an NDM-5-producing ST16 *Klebsiella pneumoniae* from a Dutch patient without travel history abroad, August 2015. *Eurosurveillance*, 20(41). <https://doi.org/10.2807/1560-7917.ES.2015.20.41.30040>

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# Isolation of an NDM-5-producing ST16 *Klebsiella pneumoniae* from a Dutch patient without travel history abroad, August 2015

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## Citation style for this article:

Bathoorn E, Rossen JW, Lokate M, Friedrich AW, Hammerum AM. Isolation of an NDM-5-producing ST16 *Klebsiella pneumoniae* from a Dutch patient without travel history abroad, August 2015. Euro Surveill. 2015;20(41):pii=30040. DOI: <http://dx.doi.org/10.2807/1560-7917.ES.2015.20.41.30040>

Article submitted on 02 October 2015 / accepted on 14 October 2015 / published on 15 October 2015

**A New Delhi Metallo-beta-lactamase-5 (NDM-5)-producing ST16 *Klebsiella pneumoniae* strain was isolated from a Dutch patient in a long-term care facility without recent travel history abroad. Core genome multilocus sequence typing (cgMLST) revealed that the Dutch isolate was clonally related to isolates detected in four patients in Denmark in 2014. Public health experts and clinicians need to be informed; repetitive screening may be needed in patients without known risk factors for carbapenemases-producing *Enterobacteriaceae* who have undergone antibiotic treatment.**

Here we report of a New Delhi Metallo-beta-lactamase-5 (NDM-5)-carrying ST16 *Klebsiella pneumoniae*, isolated from a hospitalised patient in the Netherlands, with no recent history of travel abroad. Analysis by core genome multilocus sequence typing (cgMLST) based on the core genome sequence of the isolate, showed that it is clonally related to four recently reported isolates cultured from four patients in two hospitals in Denmark, in 2014 [1].

## Background

NDM is a carbapenemase that has been detected for the first time in a Swedish patient returning from New Delhi in 2008 [2]. In the first years thereafter, introduction of NDM-producing isolates in European hospitals was associated with returning travellers from India and Pakistan [3]. NDM-producing *Enterobacteriaceae* are rapidly dispersing over the world, and cases without any epidemiological links to the Indian subcontinent have been reported [4]. Reports on detection of NDM in environmental samples and in the food chain are worrisome, in particular since this might implicate spread of these resistance genes in the community [5,6]. In northern and western Europe, however, identification

of patients with NDM-producing *Enterobacteriaceae* is still rare [7].

Currently, nine types of NDMs have been detected, of which NDM-1 is the most prevalent type [8]. NDM-5 has been isolated for the first time in the United Kingdom in 2011 from a patient returning from India [9]. Cases of NDM-5-carrying *Enterobacteriaceae* are sporadic. Some unrelated cases have been described, for instance three cases in Algeria and a case in Spain, with no recent history of travel abroad [10,11]. The identification of unrelated cases colonised with the same of NDM-5-producing *K. pneumoniae* ST16 clone in two countries with low prevalence, may be cause for concern.

## Case description

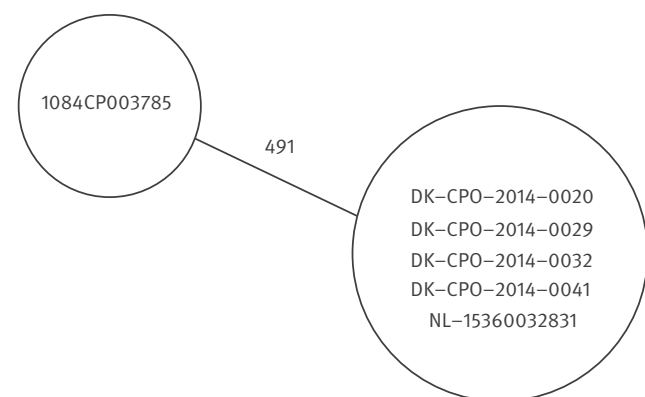
A Dutch patient in their 50s suffering from spinal cord injury had been admitted for rehabilitation in a long-term care facility in a northern region of the Netherlands. Upon admission in August 2015 (day 0), swab cultures taken from throat and rectum were negative for carbapenem-resistant Gram-negatives. The patient was treated upon admission with ceftriaxone 2 gr once per day for seven days, for an infection.

On day 10, an NDM-5-producing *K. pneumoniae* was cultured from a rectal swab taken as part of routine screening. Antibigram based on automated susceptibility testing (VITEK2, bioMérieux, Marcy l'Etoile, France) and E-tests (AB Biodisk, Mannheim, Germany) showed increased minimum inhibitory concentrations (MICs) to meropenem and imipenem, and susceptibility to gentamicin, fosfomycin, and colistin (Table).

Whole genome sequencing, de novo assembly, and assessment of multilocus sequence typing (MLST), cgMLST, and resistome were performed as described previously [12,13]. The strain harboured beta-lactam

## FIGURE

Minimal spanning tree of the four Danish NDM-5-producing ST16 *Klebsiella pneumoniae* isolates, the Dutch isolate, and reference strain *K. pneumoniae* 1084



NDM: New Delhi Metallo-beta-lactamase.

Sequences of core genome genes of Danish (DK-) isolates, the Dutch (NL-) isolate, and reference strain *K. pneumoniae* 1084 (GenBank accession number CP003785) were compared.

The tree is based on 634 columns, pairwise ignoring missing values. Number of allelic mismatches is presented.

genes *bla*<sub>NDM-5</sub>, *bla*<sub>CTX-M-15</sub>, *bla*<sub>SHV-1</sub>, *bla*<sub>OXA-1</sub>, and *bla*<sub>TEM-1b</sub>; non-beta-lactam acquired resistance genes included *strB*, *strA*, *aac(6'')lb-cr*, *QnrB66*, *fosA*, *catB3*, *sul2*, *tet(A)*, and *dfrA14*.

The patient was isolated and their room was cleaned and disinfected once a day. Throat and rectum swabs were taken twice a week from all 25 patients on the ward during the following three weeks. Pooled samples from rectum and throat were tested both by direct PCR (Check-direct CPE, Check-points, Wageningen, the Netherlands), and incubated in Brain-Heart Infusion broth with 0.25 mg/L ceftriaxone. The broth was subsequently cultured on combination Iso-Sensitest agar plates containing ceftriaxone, ceftazidime, tobramycin, or piperacillin/tazobactam (Mediaproduits, Groningen, the Netherlands). All patients were screened likewise upon admission or discharge for a period of three weeks. Ten patients who had been hospitalised on the same ward from day 0, and had already been discharged, were contacted and invited to the ward for taking cultures. Among 45 patients investigated, no additional cases have been detected as at 15 October 2015.

To search for the origin of the NDM-positive *K. pneumoniae* strain, environmental cultures were taken. All healthcare workers on the ward received a questionnaire on the following risk factors: recent stay in a foreign hospital as patient or worker, or being colonised previously with carbapenemase-producing *Enterobacteriaceae* (CPE). We did not advise to take cultures from healthcare workers because the Dutch

## TABLE

Antimicrobial minimum inhibitory concentrations for the NDM-5-producing *Klebsiella pneumoniae* isolate in a Dutch patient in a long-term care facility, August 2015

Antimicrobial agent	MIC (mg/L) <sup>a</sup>	Interpretation <sup>a</sup>
Piperacillin/tazobactam	≥ 128	S
Cefotaxime <sup>b</sup>	16	R
Ceftazidime	≥ 64	R
Cefepime	≥ 64	R
Meropenem <sup>b</sup>	6	I
Imipenem	≥ 16	R
Ciprofloxacin	≥ 64	R
Gentamicin	≤ 1	S
Tobramycin	≥ 64	R
Trimethoprim-sulfamethoxazole	≥ 320	R
Colistin	≤ 0.5	S
Tigecyclin <sup>b</sup>	2	I
Fosfomycin <sup>b</sup>	3	S

MIC: minimum inhibitory concentration; I: intermediate; R: resistant; S: susceptible.

<sup>a</sup> According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.

<sup>b</sup> Measured by E-test.

infection control guidelines recommend only to screen healthcare workers in case of ongoing transmission after implementation of outbreak control measures [14]. We did not identify any likely origins through these investigations.

Literature was searched for common sources of NDM-5-producing *Enterobacteriaceae*. A recently published report on a Danish cluster of four patients with NDM-5-producing *K. pneumoniae* clones presented a similar resistome to our case (*bla*<sub>NDM-5</sub>, *bla*<sub>CTX-M-15</sub>, *bla*<sub>SHV-1</sub>, *bla*<sub>OXA-1</sub>, and *bla*<sub>TEM-1b</sub>) [1]. Sequences of the Danish isolates were compared with the isolate from the Dutch case. All isolates belonged to ST16. The results of cgMLST analysis using a typing scheme described by Bialek-Davenet et al. [15] showed no allelic mismatches between our isolate and the Danish isolates (NCBI BioProject ID PRJNA285138). When comparing with reference strain *K. pneumoniae* 1084 (GenBank accession number CP003785), we found 491 allelic mismatches (Figure). Thus, the isolate of our patient is clonally related to the Danish isolates.

## Discussion

We report the sporadic detection of an NDM-5-producing *K. pneumoniae* from a Dutch patient with no risk factors for acquisition. The origin of the strain is unknown. Similar cases have been presented in Denmark with clonally related isolates. There were no known direct epidemiological links between the Danish patients and our case: the Danish positive cases did not travel to the Netherlands, and in our long-term care facility, no Danish patients were hospitalised during the past year.

Unknown links or a common source may be the reason for the clonality of this rare isolate.

Another explanation might be that the clonal strains have been acquired from a foodborne source. *Escherichia coli* carrying *bla*<sub>NDM-5</sub>, *bla*<sub>CTX-M</sub> and *bla*<sub>TEM</sub> have been reported as cause of mastitis in cows, suggesting that *bla*<sub>NDM-5</sub>-carrying plasmids might enter the food chain. Moreover, contamination of retail chicken meat with *K. pneumoniae* producing a combination of NDM, CTX-M-15, TEM, and SHV-1 has recently been reported [16]. Typing of *bla*<sub>NDM</sub>, plasmid, and MLST of these isolates may reveal a link with a foodborne source.

*K. pneumoniae* ST16 is a highly prevalent type causing nosocomial infections [17]. It is unsure whether this ST16 clone is newly introduced, or whether this clone was already present in our region, and has now acquired an NDM-5-carrying plasmid. We anticipate a full study, which will assess the relation between the NDM-5-producing isolates and the epidemic CTX-M-15-producing *K. pneumoniae* ST16 isolates in our region, and possible links with the food chain. Since our case was detected by chance and we did not identify any routes of transmission, further cases may be found in the Netherlands or elsewhere. We contacted national reference centers in Europe to find cases retrospectively, and to alert them about potential future cases. Finally, our case suggests that it might be necessary, under specific conditions, to screen for CPE also in patients who do not have a recent history of travel to a CPE-endemic country, and that diagnostics excluding CPE at admission, should be repeated if patients are using antibiotics during hospitalisation.

## Conflict of interest

None declared.

## Authors' contributions

EB collected the data and drafted the manuscript, JWR supervised the molecular research and analysis, ML organised infection control measurements and risk assessments, AWF participated in the coordination and concept of the manuscript, AMH coordinated and edited the manuscript.

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